ISOLATION OF BACTERIAL GENOMIC DNA

T.-C. He 7-24-05

1. Transfer 1.8 ml overnight culture to a 2-ml micro-centrifuge tube and spin 2 min. Decant the supernatant.
2. Drain well onto a Kimwipe.
3. Resuspend the pellet in 467 µl TE buffer by repeated pipetting.
4. Add 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K, mix, and incubate 1hr at 50 C.
5. Add an equal volume of phenol/chloroform and mix well by inverting the tube until the phases are completely mixed.
6. Spin 2 min.
7. Transfer the upper aqueous phase to a new tube and add an equal volume of phenol/chloroform.
8. Spin 2 min.
9. Transfer the upper aqueous phase to a new tube.
10. Add 1/10 volume of 3M sodium acetate.
11. Add 0.6 volumes of isopropanol and mix gently until the DNA precipitates.
12. Centrifuge to pellet DNA.
13. Wash in 70% Ethanol, 2x.
14. Resuspend DNA in 100-200 µl ddH2O.
15. After DNA has dissolved, measure the concentration by diluting 10 µl of DNA into 1 ml of ddH2O (1:100 dilution) and measure absorbance at 260 nm.
16. Concentration of original DNA solution in µg/ml = Abs x 100 x 50 µg/ml.

CAUTION: PHENOL CAUSES SEVERE BURNS, WEAR GLOVES GOGGLES, AND LAB COAT AND KEEP TUBES CAPPED TIGHTLY.